FILE 'MEDLINE' ENTERED AT 11:04:39 ON 19 FEB 2003
L8 111 S (EC 2.7.3.9)
L9 91 S L8 AND (ENZYME (W) I)
L10 167259 S (PHOSPH? OR PTS OR (ENZYME(W)I))/TI
L11 82 S L10 AND L9

=> d bib,abs 33,35,36,37,42,46,47,49,60,61,65,72,73,80

L11 ANSWER 33 OF 82 MEDLINE
AN 96293469 MEDLINE
DN 96293469 PubMed ID: 8692938
TI The N-terminal domain of Escherichia coli **enzyme I** of the **phosphoenolpyruvate**/glycose **phosphotransferase** system: molecular cloning and characterization.
AU Chauvin F; Fomenkov A; Johnson C R; Roseman S
CS Department of Biology, The McCollum-Prat Institute, Baltimore, MD 21218, USA.
NC GM38759 (NIGMS)
RR04328 (NCRR)
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1996 Jul 9) 93 (14) 7028-31.
Journal code: 7505876. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199608
ED Entered STN: 19960911
Last Updated on STN: 19960911
Entered Medline: 19960829
AB The bacterial phosphoenolpyruvate/glycose phosphotransferase system (PTS) comprises a group of proteins that catalyze the transfer of the phosphoryl group from phosphoenolpyruvate (PEP) to sugars concomitant with their translocation. The first two steps of the phosphotransfer sequence are PEP <--> **Enzyme I** (EI) <--> HPr (the histidine-containing phosphocarrier protein). We have proposed that many functions of the PTS are regulated by EI, which undergoes a monomer/dimer transition. EI monomer (63.5 kDa) comprises two major domains: a flexible C-terminal domain (EI-C) and a protease-resistant, structurally stable N-terminal domain (EI-N) containing the active site His. Trypsin treatment of Salmonella typhimurium EI yielded EI-N, designated EI-N(t). Homogeneous recombinant Escherichia coli EI-N [i.e., EI-N(r)], has now been prepared in quantity, shows the expected thermodynamic unfolding properties and, similarly to EI-N(t), is phosphorylated by phospho-HPr, but not by PEP. In addition, binding of EI-N(r) to HPr was studied by isothermal titration calorimetry: K/a = 1.4 x 10(5) M(-1) and delta H = +8.8 kcal x mol(-1). Both values are comparable to those for HPr binding to intact EI. Fluorescence anisotropy [dansyl-EI-N(r)] and gel filtration of EI-N(r) show that it does not dimerize. These results emphasize the role of EI-C in dimerization and the regulation of intact EI.

L11 ANSWER 35 OF 82 MEDLINE
AN 96134840 MEDLINE
DN 96134840 PubMed ID: 8555180
TI Importance of the region around glycine-338 for the activity of **enzyme I** of the Escherichia coli **phosphoenolpyruvate**:sugar **phosphotransferase** system.
AU Seok Y J; Lee B R; Gazdar C; Svenson I; Yadla N; Peterkofsky A
CS Laboratory of Biochemical Genetics, National Heart, Lung and Blood Institute, Bethesda, Maryland 20892, USA.
SO BIOCHEMISTRY, (1996 Jan 9) 35 (1) 236-42.
Journal code: 0370623. ISSN: 0006-2960.
CY United States

DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199602
ED Entered STN: 19960312
Last Updated on STN: 19960312
Entered Medline: 19960227
AB The gene encoding **enzyme I** of the phosphoenolpyruvate:sugar phosphotransferase system from an Escherichia coli **enzyme I** mutant was cloned and sequenced. The mutation was shown to be a guanine to adenine transition resulting in an altered protein in which glycine-338 was replaced by aspartic acid. The **enzyme I** structural gene was mutated to change glycine-338 to a variety of other amino acid residues. Fermentation tests indicated that glycine-338 could be mutated to alanine with no gross loss in phosphotransferase activity, while mutation to valine, glutamic acid, aspartic acid, arginine, histidine, or asparagine led to significant loss of activity. An expression vector for **enzyme I** was mutated to change glycine-338 to a variety of other amino acid residues and highly purified mutant proteins were prepared. Analysis of phosphorylation of the proteins by PEP indicated that mutation of glycine-338 to alanine had little effect on phosphorylation, mutation to valine substantially decreased phosphorylation, change to histidine or arginine drastically diminished phosphorylation, and mutation to aspartic or glutamic acids abolished phosphorylation activity. Mutation at glycine-338 influences the autophosphorylation rather than the phosphoryl transfer activity of **enzyme I**.

L11 ANSWER 36 OF 82 MEDLINE
AN 96133934 MEDLINE
DN 96133934 PubMed ID: 8552636
TI Importance of the carboxyl-terminal domain of **enzyme I** of the Escherichia coli **phosphoenolpyruvate**: sugar **phosphotransferase** system for **phosphoryl** donor specificity.
AU Seok Y J; Lee B R; Zhu P P; Peterkofsky A
CS Laboratory of Biochemical Genetics, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA.
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1996 Jan 9) 93 (1) 347-51.
Journal code: 7505876. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199602
ED Entered STN: 19960306
Last Updated on STN: 19960306
Entered Medline: 19960222
AB The first protein component of the Escherichia coli phosphoenolpyruvate: sugar phosphotransferase system (PTS) is the 64-kDa protein **enzyme I** (EI), which can be phosphorylated by phosphoenolpyruvate (PEP) and carry out phosphotransfer to the acceptor heat-stable protein (HPr). The isolated amino-terminal domain (EIN) of E. coli EI is no longer phosphorylated by PEP but retains the ability to participate in reversible phosphotransfer to HPr. An expression vector was constructed for the production of large amounts of EIN, and conditions were developed for maximal expression of the protein. A three-column procedure is described for purification to homogeneity of EIN; a 500-ml culture yields approximately 80 mg of pure protein in about a 75% yield. Intact E. coli EI is effective in phosphotransfer from PEP to HPr from E. coli but not to the HPrs from Bacillus subtilis or Mycoplasma capricolum. Phosphotransfer from EI to enzyme IIAglc (EIIAglc) from E. coli or M. capricolum requires the intermediacy of HPr. The phosphorylated form of EIN is capable of more

general phosphotransfer; it will effect phosphotransfer to HPrs from E. coli, B. subtilis, and M. capricolum as well as to EIAglc from E. coli. These studies demonstrate that the carboxyl-terminal domain of EI confers on the protein the capability to accept a phosphoryl group from PEP as well as a discriminator function that allows the intact protein to promote effective phosphoryl transfer only to E. coli HPr.

L11 ANSWER 37 OF 82 MEDLINE
AN 96102158 MEDLINE
DN 96102158 PubMed ID: 8524808
TI Coupling the **phosphotransferase** system and the methyl-accepting chemotaxis protein-dependent chemotaxis signaling pathways of Escherichia coli.
AU Lux R; Jahreis K; Bettenbrock K; Parkinson J S; Lengeler J W
CS Fachbereich Biologie/Chemie, Universitat Osnabruck, Germany.
NC GM19559 (NIGMS)
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1995 Dec 5) 92 (25) 11583-7.
Journal code: 7505876. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199601
ED Entered STN: 19960219
Last Updated on STN: 19970203
Entered Medline: 19960124
AB Chemotactic responses in Escherichia coli are typically mediated by transmembrane receptors that monitor chemoeffector levels with periplasmic binding domains and communicate with the flagellar motors through two cytoplasmic proteins, CheA and CheY. CheA autophosphorylates and then donates its phosphate to CheY, which in turn controls flagellar rotation. E. coli also exhibits chemotactic responses to substrates that are transported by the phosphoenolpyruvate (PEP)-dependent carbohydrate phosphotransferase system (PTS). Unlike conventional chemoreception, PTS substrates are sensed during their uptake and concomitant phosphorylation by the cell. The phosphoryl groups are transferred from PEP to the carbohydrates through two common intermediates, **enzyme I** (EI) and phosphohistidine carrier protein (HPr), and then to sugar-specific enzymes II. We found that in mutant strains HPr-like proteins could substitute for HPr in transport but did not mediate chemotactic signaling. In in vitro assays, these proteins exhibited reduced phosphotransfer rates from EI, indicating that the phosphorylation state of EI might link the PTS phospho-relay to the flagellar signaling pathway. Tests with purified proteins revealed that unphosphorylated EI inhibited CheA autophosphorylation, whereas phosphorylated EI did not. These findings suggest the following model for signal transduction in PTS-dependent chemotaxis. During uptake of a PTS carbohydrate, EI is dephosphorylated more rapidly by HPr than it is phosphorylated at the expense of PEP. Consequently, unphosphorylated EI builds up and inhibits CheA autophosphorylation. This slows the flow of phosphates to CheY, eliciting an up-gradient swimming response by the cell.

L11 ANSWER 42 OF 82 MEDLINE
AN 95324521 MEDLINE
DN 95324521 PubMed ID: 7601098
TI Control of glucose metabolism by the enzymes of the glucose **phosphotransferase** system in Salmonella typhimurium.
AU van der Vlag J; van't Hof R; van Dam K; Postma P W
CS E. C. Slater Instituut, University of Amsterdam, The Netherlands.
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1995 May 15) 230 (1) 170-82.
Journal code: 0107600. ISSN: 0014-2956.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)

LA English
FS Priority Journals
EM 199508
ED Entered STN: 19950822
Last Updated on STN: 19950822
Entered Medline: 19950809

AB The quantitative role of the phosphoenolpyruvate:glucose phosphotransferase system (glucose phosphotransferase system) in glucose uptake and metabolism, and phosphotransferase-system-mediated regulation of glycerol uptake, was studied in vivo in Salmonella typhimurium. Expression plasmids were constructed which contained the genes encoding **enzyme I** (ptsI), HP (ptsH), IIAGlc (crr), and IICBGlc (ptsG) of the glucose phosphotransferase system behind inducible promoters. These plasmids allowed the controlled expression of each of the glucose phosphotransferase system proteins from about 30% to about 300% of its wild-type level. When **enzyme I**, HPr or IIAGlc were modulated between 30% and 300% of their wild-type value, hardly any effects on the growth rate on glucose, the glucose oxidation rate, the rate of methyl alpha-D-glucopyranoside (a glucose analog) uptake or the phosphotransferase-system-mediated inhibition of glycerol uptake by methyl alpha-D-glucopyranoside were observed. Employing the method of metabolic control analysis, it was shown that the enzyme flux control coefficients of these phosphotransferase system components on the different measured processes were close to zero. The enzyme flux control coefficient of IICBGlc on growth on glucose or glucose oxidation was also close to zero. In contrast, the enzyme flux control coefficient of IICBGlc on the flux through the glucose phosphotransferase system (transport and phosphorylation) was 0.72. The experimentally determined enzyme flux control coefficients allowed us to calculate the flux control coefficients of the phosphoenolpyruvate/pyruvate and methyl alpha-D-glucopyranoside/methyl alpha-D-glucopyranoside 6-phosphate couples and the process control coefficients of the phosphotransfer reactions of the glucose phosphotransferase system. We discuss the implications of these values and the possible control points in the glucose phosphotransferase system.

L11 ANSWER 46 OF 82 MEDLINE
AN 94178918 MEDLINE
DN 94178918 PubMed ID: 8132321
TI Sequence and expression of the genes for HPr (ptsH) and **enzyme I** (ptsI) of the **phosphoenolpyruvate**-dependent **phosphotransferase** transport system from Streptococcus mutans.
AU Boyd D A; Cvitkovitch D G; Hamilton I R
CS Department of Oral Biology, University of Manitoba, Winnipeg, Canada.
SO INFECTION AND IMMUNITY, (1994 Apr) 62 (4) 1156-65.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-L15191
EM 199404
ED Entered STN: 19940428
Last Updated on STN: 19940428
Entered Medline: 19940421

AB We report the sequencing of a 2,242-bp region of the Streptococcus mutants NG5 genome containing the genes for ptsH and ptsI, which encode HPr and **enzyme I** (EI), respectively, of the phosphoenolpyruvate-dependent phosphotransferase transport system. The sequence was obtained from two cloned overlapping genomic fragments; one expresses HPr and a truncated EI, while the other expresses a full-length EI in Escherichia coli, as determined by Western immunoblotting. The ptsI gene appeared to be expressed from a region located in the ptsH gene. The S. mutans NG5 pts operon does not appear to be linked to other phosphotransferase transport

system proteins as has been found in other bacteria. A positive fermentation pattern on MacConkey-glucose plates by an E. coli ptsI mutant harboring the S. mutans NG5 ptsI gene on a plasmid indicated that the S. mutans NG5 EI can complement a defect in the E. coli gene. This was confirmed by protein phosphorylation experiments with 32P-labeled phosphoenolpyruvate indicating phosphotransfer from the S. mutans NG5 EI to the E. coli HPr. Two forms of the cloned EI, both truncated to varying degrees in the C-terminal region, were inefficiently phosphorylated and unable to complement fully the ptsI defect in the E. coli mutant. The deduced amino acid sequence of HPr shows a high degree of homology, particularly around the active site, to the same protein from other gram-positive bacteria, notably, S. salivarius, and to a lesser extent with those of gram-negative bacteria. The deduced amino acid sequence of S. mutans NG5 EI also shares several regions of homology with other sequenced EIs, notably, with the region around the active site, a region that contains the only conserved cystidyl residue among the various proteins and which may be involved in substrate binding.

L11 ANSWER 47 OF 82 MEDLINE
AN 94124012 MEDLINE
DN 94124012 PubMed ID: 8294015
TI **Phosphotransferase** system of Streptococcus salivarius: characterization of the ptsH gene and its product.
AU Gagnon G; Vadeboncoeur C; Frenette M
CS Departement de Biochimie (Sciences), Universite Laval, Quebec, Canada.
SO GENE, (1993 Dec 22) 136 (1-2) 27-34.
Journal code: 7706761. ISSN: 0378-1119.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-L08627; GENBANK-L15362; GENBANK-L15363; GENBANK-L24529; GENBANK-X72987; GENBANK-X72988; GENBANK-X72989; GENBANK-Z15047; GENBANK-Z15048; GENBANK-Z17217
EM 199403
ED Entered STN: 19940314
Last Updated on STN: 19950206
Entered Medline: 19940301
AB The Streptococcus salivarius ptsH gene encoding histidine-containing phosphocarrier protein (HPr) of the phosphotransferase system (PTS) has been cloned, sequenced, and found to be part of a ptsH, ptsI operon. Upstream from ptsH, putative -35 and -10 boxes and a Shine-Dalgarno sequence highly similar to the Escherichia coli consensus regulatory elements were identified. A second promoter, located in the ptsH coding sequence was also observed and is sufficient for the expression of the S. salivarius ptsI gene, encoding **enzyme I** of the PTS in E. coli [Gagnon et al., Gene 121 (1992) 71-78]. The amino acid sequence of S. salivarius HPr, inferred from the ptsH sequence, shared identity varying between 37 and 76% with known HPr from other bacteria. Moreover, the S. salivarius HPr shared 78% identity with an HPr-like protein of Aspergillus fumigatus, a eukaroytic mold that does not possess a functional PTS. Expression analysis of S. salivarius HPr in E. coli demonstrated that (i) S. salivarius ptsH is expressed in E. coli under the control of its own promoter, (ii) S. salivarius HPr synthesized by E. coli is completely processed by methionine aminopeptidase, and (iii) S. salivarius HPr is phosphorylated in vivo by E. coli **enzyme I**. It was also observed that, in E. coli, the copy number of pUC18 bearing S. salivarius ptsH was reduced more than 25-fold, as compared to pUC18 without an insertion.

L11 ANSWER 49 OF 82 MEDLINE
AN 92385033 MEDLINE
DN 92385033 PubMed ID: 1515124
TI Properties of **phosphorylated** protein intermediates of the

bacterial **phosphoenolpyruvate**:sugar **phosphotransferase** system.
AU Anderson J W; Waygood E B; Saier M H Jr; Reizer J
CS Department of Biochemistry, University of Saskatchewan, Saskatoon, Canada.
NC 2 RO1 AI 14176 (NIAID)
5 RO1 AI 21702 (NIAID)
SO BIOCHEMISTRY AND CELL BIOLOGY, (1992 Mar-Apr) 70 (3-4) 242-6.
Journal code: 8606068. ISSN: 0829-8211.
CY Canada
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199210
ED Entered STN: 19921023
Last Updated on STN: 19921023
Entered Medline: 19921007
AB The phosphohydrolysis properties of the following phosphoprotein intermediates of the bacterial phosphoenolpyruvate:sugar phosphotransferase system (PTS) were investigated: **enzyme I**, HPr, and the IIAGlc domain of the glucose enzyme II of Bacillus subtilis; and IIAGlc (fast and slow forms) of Escherichia coli. The phosphohydrolysis properties were also studied for the site-directed mutant H68A of B. subtilis IIA Glc. Several conclusions were reached. (i) The phosphohydrolysis properties of the homologous phosphoprotein intermediates of B. subtilis and E. coli are similar. (ii) These properties deviate from those of isolated N delta 1- and N epsilon 2-phosphohistidine indicating the participation of neighbouring residues at the active sites of these proteins. (iii) The rates of phosphohydrolysis of the H68A mutant of B. subtilis IIAGlc were reduced compared with the wild-type protein, suggesting that both His-83 and His-68 are present at the active site of wild-type IIAGlc. (iv) The removal of seven N-terminal residues of E. coli IIAGlc reduced the rates of phosphohydrolysis between pH 5 and 8.

L11 ANSWER 60 OF 82 MEDLINE
AN 87109090 MEDLINE
DN 87109090 PubMed ID: 3542977
TI Genetic expression of **enzyme I** activity of the **phosphoenolpyruvate**:sugar **phosphotransferase** system in ptsHI deletion strains of Salmonella typhimurium.
AU Chin A M; Sutrina S; Feldheim D A; Saier M H Jr
NC 2 RO1 AI 14176-09A1 (NIAID)
5 RO1 AI 21702 (NIAID)
SO JOURNAL OF BACTERIOLOGY, (1987 Feb) 169 (2) 894-6.
Journal code: 2985120R. ISSN: 0021-9193.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198703
ED Entered STN: 19900303
Last Updated on STN: 19970203
Entered Medline: 19870313
AB Mutants expressing a novel **enzyme I** of the phosphoenolpyruvate:sugar phosphotransferase system, termed **enzyme I**, were isolated from strains of Salmonella typhimurium which were deleted for the HPr and **enzyme I** structural genes. The mutations lay in a newly defined gene, termed ptsJ, which mapped on the S. typhimurium chromosome between the ptsHI operon and the cysA gene.

L11 ANSWER 61 OF 82 MEDLINE
AN 87008576 MEDLINE
DN 87008576 PubMed ID: 3020035
TI **Phosphate** transfer between acetate kinase and **enzyme**

I of the bacterial **phosphotransferase** system.
AU Fox D K; Meadow N D; Roseman S
NC 5T32GM-07231 (NIGMS)
CA21901 (NCI)
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1986 Oct 15) 261 (29) 13498-503.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198611
ED Entered STN: 19900302
Last Updated on STN: 19970203
Entered Medline: 19861117
AB Interactions between homogeneous acetate kinase and proteins of the phosphoenolpyruvate:glucose phosphotransferase system (PTS) were studied. The phosphorylation of D-glucose was followed spectrophotometrically using a coupled assay system, and acetate kinase and GTP were found to substitute for phosphoenolpyruvate provided that each of the PTS proteins was present in the mixture. To further define the phosphoryl transfer reaction pathway, the system was simplified to include only the homogeneous, soluble PTS proteins. 32P was transferred from [gamma-32P]ATP to the protein IIIGlc, but this transfer reaction required acetate kinase, and the PTS proteins **Enzyme I** and HPr. These results suggested that acetate kinase interacts with the first protein in the PTS sequence, **Enzyme I**. Acetate kinase was therefore incubated with [32P] phospho-**Enzyme I**, and a direct transfer of the phosphoryl group was observed without the addition of any other protein. These results show that there is a reversible transfer of the phosphoryl group between **Enzyme I** and acetate kinase. The possible role of this interaction in regulating sugar uptake by the Krebs cycle is discussed.

L11 ANSWER 65 OF 82 MEDLINE
AN 85199851 MEDLINE
DN 85199851 PubMed ID: 3922407
TI **Phosphoenolpyruvate**-dependent protein kinase **enzyme** I of Streptococcus faecalis: purification and properties of the enzyme and characterization of its active center.
AU Alpert C A; Frank R; Stuber K; Deutscher J; Hengstenberg W
SO BIOCHEMISTRY, (1985 Feb 12) 24 (4) 959-64.
Journal code: 0370623. ISSN: 0006-2960.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198506
ED Entered STN: 19900320
Last Updated on STN: 19900320
Entered Medline: 19850626
AB **Enzyme I**, the phosphoenolpyruvate:protein phosphotransferase (**EC 2.7.3.9**), which is part of the bacterial phosphoenolpyruvate- (PEP) dependent phosphotransferase system, has been purified from Streptococcus faecalis by using a large-scale preparation. Size exclusion chromatography revealed a molecular weight of 140 000. On sodium dodecyl sulfate gels, **enzyme I** gave one band with a molecular weight of 70 000, indicating that **enzyme I** consists of two identical subunits. The first 59 amino acids of the amino-terminal part of the protein have been sequenced. It showed some similarities with **enzyme I** of Salmonella typhimurium. The active center of **enzyme I** has also been determined. After phosphorylation with [32P]PEP, the enzyme was cleaved by using different proteases. Labeled peptides were isolated by high-performance liquid chromatography

on a reversed-phase column. The amino acid composition or amino acid sequence of the peptides has been determined. The largest labeled peptide was obtained with Lys-C protease and had the following sequence: -Ala-Phe-Val-Thr-Asp-Ile-Gly- Gly-Arg-Thr-Ser-His*-Ser-Ala-Ile-Met-Ala-Arg-Ser-Leu-Glu-Ile-Pro-Ala- Ile-Val-Gly-Thr-Lys-. It has previously been shown that the phosphoryl group is bound to the N-3 position of a histidyl residue in phosphorylated **enzyme I**. The single His in position 12 of the above peptide must therefore carry the phosphoryl group.

L11 ANSWER 72 OF 82 MEDLINE
AN 83206164 MEDLINE
DN 83206164 PubMed ID: 6406017
TI Determination of the levels of HPr and **enzyme I** of the **phosphoenolpyruvate**-sugar **phosphotransferase** system in Escherichia coli and Salmonella typhimurium.
AU Mattoo R L; Waygood E B
SO CANADIAN JOURNAL OF BIOCHEMISTRY AND CELL BIOLOGY, (1983 Jan) 61 (1) 29-37.
Journal code: 8302763. ISSN: 0714-7511.
CY Canada
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198307
ED Entered STN: 19900319
Last Updated on STN: 19980206
Entered Medline: 19830715
AB The levels of histidine-containing protein HPr and **enzyme I** of the phosphoenolpyruvate-sugar phosphotransferase system of Escherichia coli strains 1100, NC3, W3110, and P650 and Salmonella typhimurium strains SB3507 and LJ144 have been determined by quantitative sugar phosphorylation assay and immunochemically. The levels have been determined for cells grown on minimal salts with glucose, fructose, mannitol, glycerol, and lactate and on nutrient broth. All determinations indicate a two- to three-fold change in the levels of **enzyme I** and HPr between growth on hexoses, which gave the higher levels, and the other growth substrates. The highest levels were not always found in glucose-grown cells. Antibodies were produced in rabbits using purified proteins from E. coli P650. The activity measurements and immunochemically determined **enzyme I** protein gave specific activities in the crude extracts of E. coli strains which were similar to that of the pure enzyme. The wild-type S. typhimurium **enzyme I** in crude extracts did not have the same immunochemical reactivity, although there was a considerable cross-reaction and the specific activity appeared to be half that of pure **enzyme I**. The HPr from both E. coli and S. typhimurium behaved identically and, although the immunoprecipitation was weak, it did indicate that HPr assays may not be as reliable as the **enzyme I** assays. The relative amounts of **enzyme I** and HPr found indicate that there are between 10- and 20-fold more HPr molecules in a cell than **enzyme I** subunits which form active dimers.

L11 ANSWER 73 OF 82 MEDLINE
AN 83114572 MEDLINE
DN 83114572 PubMed ID: 6759860
TI **Enzyme I** from salmonella typhimurium.
AU Kukuruzinska M A; Weigel N; Waygood E B
NC CA 21901 (NCI)
SO METHODS IN ENZYMOLOGY, (1982) 90 Pt E 431-6.
Journal code: 0212271. ISSN: 0076-6879.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English

FS Priority Journals
EM 198303
ED Entered STN: 19900318
Last Updated on STN: 19970203
Entered Medline: 19830311

L11 ANSWER 80 OF 82 MEDLINE
AN 82167885 MEDLINE
DN 82167885 PubMed ID: 7040430
TI Evidence for the functional association of **enzyme I** and HPr of the **phosphoenolpyruvate**-sugar **phosphotransferase** system with the membrane in sealed vesicles of Escherichia coli.
AU Saier M H Jr; Cox D F; Feucht B U; Novotny M J
NC 1 RO1 AI 14176-01 (NIAID)
SO JOURNAL OF CELLULAR BIOCHEMISTRY, (1982) 18 (2) 231-8.
Journal code: 8205768. ISSN: 0730-2312.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198206
ED Entered STN: 19900317
Last Updated on STN: 19970203
Entered Medline: 19820614
AB Several independent assay procedures were used to estimate the activities of the enzyme constituents of the phosphoenolpyruvate-sugar phosphotransferase system (PTS) in osmotically shocked bacterial membrane vesicles. The soluble enzymes of the system were found to be in association with the membrane by several criteria. Phosphoenolpyruvate-dependent sugar phosphorylation was catalyzed by this membrane-bound enzyme system far more efficiently than by a mixture of the individual enzymes at corresponding concentrations. By contrast, the rates of the phosphoryl exchange reactions catalyzed by **enzyme I** and the enzyme II complexes were essentially the same for the associated and dissociated forms of the system. Functional association of the PTS-enzyme complex was stabilized by Mg++ and phosphoenolypyruvate and could be destroyed by detergent treatment, sonication, or by passage of the vesicle preparation through a French pressure cell. These results lead to the possibility that in the intact bacterial cell the soluble enzymes of the phosphotransferase system exist, in part, as peripheral membrane constituents associated with the integral membrane enzyme II complexes.

=> d bib,abs 20-30

L11 ANSWER 20 OF 82 MEDLINE
AN 1999398438 MEDLINE
DN 99398438 PubMed ID: 10467175
TI **Phosphocarrier** proteins in an intracellular symbiotic bacterium of aphids.
AU Matsumoto K; Morioka M; Ishikawa H
CS Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo, 113-0033, Japan.
SO JOURNAL OF BIOCHEMISTRY, (1999 Sep) 126 (3) 578-83.
Journal code: 0376600. ISSN: 0021-924X.
CY Japan
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-AB025229
EM 200002
ED Entered STN: 20000229
Last Updated on STN: 20000229

Entered Medline: 20000211

AB A GroEL homolog produced by Buchnera, an intracellular symbiotic bacterium of aphids, is not only a molecular chaperone but also a novel phosphocarrier protein, suggesting that this protein plays a role in a signal transducing system specific to bacteria living in an intracellular environment. This prompted us to look into phosphocarrier proteins of Buchnera that may be shared in common with other bacteria. As a result, no evidence was obtained for the presence of sensor kinases of the two-component system in Buchnera, which are found in many bacteria. It is possible that the lack of sensor kinases is compensated for by the mulitifunctional GroEL homolog in this symbiotic bacteria. In contrast, we successfully identified three phosphotransferase system genes, ptsH, ptsI, and crr in Buchnera, and provide evidence for their active expression. While the deduced amino acid sequences of these gene products, histidine-containing phosphocarrier protein, **Enzyme I**, and Enzyme III were similar to their counterparts in Escherichia coli, the predicted isoelectric points of the Buchnera proteins were strikingly higher. It was also suggested that Buchnera **Enzyme I**, when produced in E. coli, is able to accept the phosphoryl group from phosphoenolpyruvate, but not from ATP.

L11 ANSWER 21 OF 82 MEDLINE
AN 1999157601 MEDLINE
DN 99157601 PubMed ID: 10048041
TI Regulation of the activity of the Bacillus subtilis antiterminator LicT by multiple PEP-dependent, **enzyme I**- and HPr-catalysed **phosphorylation**.
AU Lindner C; Galinier A; Hecker M; Deutscher J
CS Institut de Biologie et Chimie des Proteines, Centre National de la Recherche Scientifique, UPR412, Lyon, France.
SO MOLECULAR MICROBIOLOGY, (1999 Feb) 31 (3) 995-1006.
Journal code: 8712028. ISSN: 0950-382X.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199905
ED Entered STN: 19990517
Last Updated on STN: 19990517
Entered Medline: 19990506

AB The transcriptional antiterminator LicT regulates the induction and carbon catabolite repression of the Bacillus subtilis bglPH operon. LicT is inactive in mutants affected in one of the two general components of the phosphoenolpyruvate (PEP):glycose phosphotransferase system, **enzyme I** or histidine-containing protein (HPr). We demonstrate that LicT becomes phosphorylated in the presence of PEP, **enzyme I** and HPr. The phosphoryl group transfer between HPr and LicT is reversible. Phosphorylation of LicT with PEP, **enzyme I** and HPr led to the appearance of three additional LicT bands on polyacrylamide-urea gels. These bands probably correspond to one-, two- and threefold phosphorylated LicT. After phosphorylation of LicT with [32P]-PEP, **enzyme I** and HPr, proteolytic digestion of [32P]-P-LicT, separation of the peptides by reverse-phase chromatography, mass spectrometry and N-terminal sequencing of radiolabelled peptides, three histidyl residues were found to be phosphorylated in LicT. These three histidyl residues (His-159, His-207 and His-269) are conserved in most members of the BglG/SacY family of transcriptional antiterminators. Phosphorylation of LicT in the presence of serylphosphorylated HPr (P-Ser-HPr) was much slower compared with its phosphorylation in the presence of HPr. The slower phosphorylation in the presence of P-Ser-HPr leading to reduced LicT activity is presumed to play a role in a recently described LicT-mediated CcpA-independent carbon catabolite repression mechanism operative for the bglPH operon.

L11 ANSWER 22 OF 82 MEDLINE
AN 1999140298 MEDLINE
DN 99140298 PubMed ID: 10048929
TI Solution structure of the 40,000 Mr **phosphoryl** transfer complex between the N-terminal domain of **enzyme I** and HPr.
AU Garrett D S; Seok Y J; Peterkofsky A; Gronenborn A M; Clore G M
CS Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0520, USA.
SO NATURE STRUCTURAL BIOLOGY, (1999 Feb) 6 (2) 166-73.
Journal code: 9421566. ISSN: 1072-8368.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS PDB-3EZA; PDB-3EZB; PDB-3EZC; PDB-3EZD; PDB-R3EZAMR
EM 199902
ED Entered STN: 19990311
Last Updated on STN: 20000303
Entered Medline: 19990225
AB The solution structure of the first protein-protein complex of the bacterial phosphoenolpyruvate: sugar phosphotransferase system between the N-terminal domain of **enzyme I** (EIN) and the histidine-containing phosphocarrier protein HPr has been determined by NMR spectroscopy, including the use of residual dipolar couplings that provide long-range structural information. The complex between EIN and HPr is a classical example of surface complementarity, involving an essentially all helical interface, comprising helices 2, 2', 3 and 4 of the alpha-subdomain of EIN and helices 1 and 2 of HPr, that requires virtually no changes in conformation of the components relative to that in their respective free states. The specificity of the complex is dependent on the correct placement of both van der Waals and electrostatic contacts. The transition state can be formed with minimal changes in overall conformation, and is stabilized in favor of phosphorylated HPr, thereby accounting for the directionality of phosphoryl transfer.

L11 ANSWER 23 OF 82 MEDLINE
AN 1998394976 MEDLINE
DN 98394976 PubMed ID: 9726852
TI Cloning and expression of the Listeria monocytogenes scott A ptsH and ptsI genes, coding for HPr and **enzyme I**, respectively, of the **phosphotransferase** system.
AU Christensen D P; Benson A K; Hutkins R W
CS School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska 68583-0919, USA.
SO APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1998 Sep) 64 (9) 3147-52.
Journal code: 7605801. ISSN: 0099-2240.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-AF030824
EM 199810
ED Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981030
AB The phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS) utilizes high-energy phosphate present in PEP to drive the uptake of several different carbohydrates in bacteria. In order to examine the role of the PTS in the physiology of Listeria monocytogenes, we identified the ptsH and ptsI genes encoding the HPr and **enzyme I** proteins, respectively, of the PTS. Nucleotide sequence analysis indicated that the predicted proteins are nearly 70% similar to HPr and **enzyme I** proteins from other organisms. Purified L.

monocytogenes HPr overexpressed in Escherichia coli was also capable of complementing an HPr defect in heterologous extracts of Staphylococcus aureus ptsH mutants. Additional studies of the transcriptional organization and control indicated that the ptsH and ptsI genes are organized into a transcription unit that is under the control of a consensus-like promoter and that expression of these genes is mediated by glucose availability and pH or by by-products of glucose metabolism.

L11 ANSWER 24 OF 82 MEDLINE
AN 1998353635 MEDLINE
DN 98353635 PubMed ID: 9689210
TI Novel **phosphotransferase** system genes revealed by bacterial genome analysis: the complete complement of **pts** genes in mycoplasma genitalium.
AU Reizer J; Paulsen I T; Reizer A; Titgemeyer F; Saier M H Jr
CS Department of Biology, University of California at San Diego, La Jolla, USA.
NC 5RO1 AI21702 (NIAID)
SO MICROBIAL AND COMPARATIVE GENOMICS, (1996) 1 (3) 151-64.
Journal code: 9616596. ISSN: 1090-6592.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199808
ED Entered STN: 19980903
Last Updated on STN: 19980903
Entered Medline: 19980826
AB The complete sequence of the Mycoplasma genitalium chromosome has recently been determined. We here report analyses of the genes encoding proteins of the phosphoenolpyruvate:sugar phosphotransferase system, PTS. These genes encode (1) **Enzyme I**, (2) HPr, (3) a glucose-specific Enzyme IICBA, (4) an inactive glucose-specific Enzyme IIB, lacking the active site cysteyl residue, and (5) a fructose-specific Enzyme IIABC. Some of the unique features of these genes and their enzyme products are as follows. (1) Each of the genes is encoded within a distinct operon. (2) Both **Enzyme I** and HPr have basic isoelectric points. (3) The glucose-specific Enzyme IIC bears a centrally located, hydrophilic, 200 amino acyl residue insert that lacks sequence similarity with any protein in the current database. (4) The fructose-specific Enzyme II has a domain order (IIABC), different from those of previously characterized fructose permeases, and its IIA domain more closely resembles the IIANtr protein of Escherichia coli than other fructose-specific IIA domains. The potential significance of these novel features is discussed.

L11 ANSWER 25 OF 82 MEDLINE
AN 1998337942 MEDLINE
DN 98337942 PubMed ID: 9671705
TI In vivo and in vitro complementation of the N-terminal domain of **enzyme I** of the Escherichia coli **phosphotransferase** system by the cloned C-terminal domain.
AU Fomenkov A; Valiakhmetov A; Brand L; Roseman S
CS Department of Biology and the McCollum-Pratt Institute, The Johns Hopkins University, Baltimore, MD 21218, USA.
NC GM11632 (NIGMS)
GM38759 (NIGMS)
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 Jul 21) 95 (15) 8491-5.
Journal code: 7505876. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals

EM 199808
ED Entered STN: 19980828
Last Updated on STN: 19980828
Entered Medline: 19980820
AB **Enzyme I** (EI) is the first protein in the phosphoryl transfer sequence from phosphoenolpyruvate (PEP) to sugar in carbohydrate uptake via the bacterial PEP:glycose phosphotransferase system. The EI monomer/dimer transition may regulate the phosphotransferase system because only the EI dimer is autophosphorylated by PEP. We previously showed that the EI monomer comprises two major domains: (i) a compact, protease-resistant N-terminal domain (EI-N), containing the active site His, and (ii) a flexible, protease-sensitive C-terminal domain (EI-C), which is required for EI dimerization. EI-N interacts with the second protein, HPr, and phospho-HPr, but EI-N neither dimerizes nor is phosphorylated by PEP. We report here the molecular cloning and some properties of EI-C. EI-C is rapidly proteolyzed in vivo. Therefore, two different overexpression vectors encoding fusion proteins were constructed. Fusion Xa contains MalE (the maltose-binding protein), the four-amino acid sequence required by protease factor Xa, followed by EI-C. Fusion G contains His-Tyr between MalE and EI-C and is cleaved by the protease genenase. Homogenous EI-C was isolated from fusion G. [32P]PEP phosphorylated EI-N when supplemented with EI-C, fusion Xa, or fusion G. EI-C may act catalytically. Complementation was also demonstrated in vivo. An Escherichia coli ptsI deletion grew on mannitol as the sole source of carbon after it was transformed with two compatible vectors; one vector encoded EI-N and the other encoded fusion Xa or fusion G. The molecular details underlying important properties of EI can now be studied.

L11 ANSWER 26 OF 82 MEDLINE
AN 1998321639 MEDLINE
DN 98321639 PubMed ID: 9660202
TI Identification of peptides inhibiting **enzyme I** of the bacterial **phosphotransferase** system using combinatorial cellulose-bound peptide libraries.
AU Mukhija S; Germeroth L; Schneider-Mergener J; Erni B
CS Departement fur Chemie und Biochemie, Universitat Bern, Switzerland.
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1998 Jun 1) 254 (2) 433-8.
Journal code: 0107600. ISSN: 0014-2956.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199807
ED Entered STN: 19980731
Last Updated on STN: 19980731
Entered Medline: 19980721
AB The phosphoenolpyruvate(P-pyruvate)-dependent sugar phosphotransferase system (PTS) is a transport and signal-transduction system which is almost ubiquitous in bacteria but does not occur in eucaryotes. It catalyzes the uptake and phosphorylation of carbohydrates and is involved in signal transduction, e.g. catabolite repression, chemotaxis, and allosteric regulation of metabolic enzymes and transporters. EI (**Enzyme I** of the PTS) is the first and central component of the divergent PTS (P-pyruvate-dependent sugar phosphotransferase system) phosphorylation cascade. Using immobilized combinatorial peptide libraries and phosphorimaging, heptapeptides and octapeptides were identified which selectively inhibit EI in vitro. The IC50 of the best peptides is 30 microM which is close to the K(M) (6 microM) of EI for its natural substrate HPr (histidine containing phosphoryl carrier protein of the PTS). The affinity-selected peptides are better inhibitors than a peptide with the active-site sequence of HPr. The selected peptides contain several basic residues and one aromatic residue which do not occur in the active site of HPr. The large proportion of basic residues most likely reflects charge complementarity to the strongly acidic active-site pocket

of EI. Guanidino groups might facilitate by complexation of the phosphoryl group the slow phosphorylation of the peptide.

L11 ANSWER 27 OF 82 MEDLINE
AN 1998244849 MEDLINE
DN 98244849 PubMed ID: 9578555
TI **Phosphorylation** destabilizes the amino-terminal domain of **enzyme I** of the Escherichia coli **phosphoenolpyruvate**:sugar **phosphotransferase** system.
AU Nosworthy N J; Peterkofsky A; Konig S; Seok Y J; Szczepanowski R H; Ginsburg A
CS Section on Protein Chemistry, Laboratory of Biochemistry, National Heart, Lung, and Blood Institute, Bethesda, Maryland 20892, USA.
SO BIOCHEMISTRY, (1998 May 12) 37 (19) 6718-26.
Journal code: 0370623. ISSN: 0006-2960.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199806
ED Entered STN: 19980611
Last Updated on STN: 19980611
Entered Medline: 19980604
AB Thermal stabilities of **enzyme I** (63 562 M(r) subunit, in the Escherichia coli phosphoenolpyruvate (PEP):sugar phosphotransferase system (PTS), and a cloned amino-terminal domain of **enzyme I** (EIN; 28 346 Mr) were investigated by differential scanning calorimetry (DSC) and far-UV circular dichroism (CD) at pH 7.5. EIN expressed in a delta pts E. coli strain showed a single, reversible, two-state transition with Tm = 57 degrees C and an unfolding enthalpy of approximately 140 kcal/mol. In contrast, monomeric EIN expressed in a wild-type strain (pts+) had two endotherms with Tm congruent with 50 and 57 degrees C and overall delta H = 140 kcal/mol and was converted completely to the more stable form after five DSC scans from 10 to 75 degrees C (without changes in CD: approximately 58% alpha-helices). Thermal conversion to a more stable form was correlated with dephosphorylation of EIN by mass spectral analysis. Dephospho-**enzyme I** (monomer right arrow over left arrow dimer) exhibited endotherms for C- and N-terminal domain unfolding with Tm = 41 and 54 degrees C, respectively. Thermal unfolding of the C-terminal domain occurred over a broad temperature range (approximately 30-50 degrees C), was scan rate- and concentration-dependent, coincident with a light scattering decrease and Trp residue exposure, and independent of phosphorylation. Reversible thermal unfolding of the nonphosphorylated N-terminal domain was more cooperative, occurring from 50 to 60 degrees C. DSC of partially phosphorylated **enzyme I** indicated that the amino-terminal domain was destabilized by phosphorylation (from Tm = 54 to approximately 48 degrees C). A decrease in conformational stability of the amino-terminal domain of **enzyme I** produced by phosphorylation of the active-site His 189 has the physiological consequence of promoting phosphotransfer to the phosphocarrier protein, HP(r).

L11 ANSWER 28 OF 82 MEDLINE
AN 97263731 MEDLINE
DN 97263731 PubMed ID: 9109646
TI Identification by NMR of the binding surface for the histidine-containing **phosphocarrier** protein HPr on the N-terminal domain of **enzyme I** of the Escherichia coli **phosphotransferase** system.
AU Garrett D S; Seok Y J; Peterkofsky A; Clore G M; Gronenborn A M
CS Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland 20892, USA.
SO BIOCHEMISTRY, (1997 Apr 15) 36 (15) 4393-8.

Journal code: 0370623. ISSN: 0006-2960.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS PDB-1EZA; PDB-1POH
EM 199705
ED Entered STN: 19970523
Last Updated on STN: 19970523
Entered Medline: 19970512
AB The interaction between the approximately 30 kDa N-terminal domain of **enzyme I** (EIN) and the approximately 9.5 kDa histidine-containing phosphocarrier protein HPr of the Escherichia coli phosphoenolpyruvate:sugar phosphotransferase system has been investigated by heteronuclear magnetic resonance spectroscopy. The complex is in fast exchange, permitting us to follow the chemical shift changes of the backbone NH and 15N resonances of EIN upon complex formation by recording a series of 1H-15N correlation spectra of uniformly 15N-labeled EIN in the presence of increasing amounts of HPr at natural isotopic abundance. The equilibrium association constant derived from analysis of the titration data is approximately 1.5 x 10(5) M(-1), and the lower limit for the dissociation rate constant is 1100 s(-1). By mapping the backbone chemical shift perturbations on the three-dimensional solution structure of EIN [Garrett, D. S., Seok, Y.-J., Liao, D.-I., Peterkofsky, A., Gronenborn, A. M., & Clore, G. M. (1997) Biochemistry 36, 2517-2530], we have identified the binding surface of EIN in contact with HPr. This surface is primarily located in the alpha domain and involves helices H1, H2, and H4, as well as the hinge region connecting helices H2 and H2'. The data also indicate that the active site His 15 of HPr must approach the active site His 189 of EIN along the shallow depression at the interface of the alpha and alpha/beta domains. Interestingly, both the backbone and side chain resonances (assigned from a long-range 1H-15N correlation spectrum) of His 189, which is located at the N-terminus of helix H6 in he alpha/beta domain, are only minimally perturbed upon complexation, indicating that His 189 (in the absence of phosphorylation) does not undergo any significant conformational change or change in pK(a) value upon HPr binding. On the basis of results of this study, as well as a previous study which delineated the interaction surface for EI on HPr [van Nuland, N. A. J., Boelens, R., Scheek, R. M., & Robillard, G. T. (1995) J. Mol. Biol. 246, 180-193], a model for the EIN/HPr complex is proposed in which helix 1 (residues 16-27) and the helical loop (residues 49-53) of HPr slip between the two pairs of helices constituting the alpha domain of EIN. In addition, we suggest a functional role for the kink between helices H2 and H2' of EIN, providing a flexible joint for this interaction to take place.

L11 ANSWER 29 OF 82 MEDLINE
AN 97239066 MEDLINE
DN 97239066 PubMed ID: 9084757
TI **Enzyme I**: the first protein and potential regulator of the bacterial **phosphoenolpyruvate**: glycose **phosphotransferase** system.
AU Chauvin F; Brand L; Roseman S
CS Department of Biology, Johns Hopkins University, Baltimore, MD 21218, USA.
NC GM11632 (NIGMS)
GM38759 (NIGMS)
GM51215 (NIGMS)
SO RESEARCH IN MICROBIOLOGY, (1996 Jul-Sep) 147 (6-7) 471-9. Ref: 24
Journal code: 8907468. ISSN: 0923-2508.
CY France
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals

EM 199704
ED Entered STN: 19970422
Last Updated on STN: 19970422
Entered Medline: 19970408

L11 ANSWER 30 OF 82 MEDLINE
AN 97207064 MEDLINE
DN 97207064 PubMed ID: 9054557
TI Solution structure of the 30 kDa N-terminal domain of **enzyme I** of the Escherichia coli **phosphoenolpyruvate**:sugar **phosphotransferase** system by multidimensional NMR.
AU Garrett D S; Seok Y J; Liao D I; Peterkofsky A; Gronenborn A M; Clore G M
CS Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland 20892, USA.
SO BIOCHEMISTRY, (1997 Mar 4) 36 (9) 2517-30.
Journal code: 0370623. ISSN: 0006-2960.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS PDB-1EZA; PDB-1EZB; PDB-1EZC; PDB-R1EZAMR; PDB-R1YZAMR
EM 199703
ED Entered STN: 19970407
Last Updated on STN: 19970407
Entered Medline: 19970326
AB The three-dimensional solution structure of the 259-residue 30 kDa N-terminal domain of **enzyme I** (EIN) of the phosphoenolpyruvate:sugar phosphotransferase system of Escherichia coli has been determined by multidimensional nuclear magnetic resonance spectroscopy. **Enzyme I**, which is autophosphorylated by phosphoenolpyruvate, reversibly phosphorylates the phosphocarrier protein HPr, which in turn phosphorylates a group of membrane-associated proteins, known as enzymes II. To facilitate and confirm NH, 15N, and 13C assignments, extensive use was made of perdeuterated 15N- and 15N/13C-labeled protein to narrow line widths. Ninety-eight percent of the 1H, 15N, and 13C assignments for the backbone and first side chain atoms of protonated EIN were obtained using a combination of double and triple resonance correlation experiments. The structure determination was based on a total of 4251 experimental NMR restraints, and the precision of the coordinates for the final 50 simulated annealing structures is 0.79 +/- 0.18 A for the backbone atoms and 1.06 +/- 0.15 A for all atoms. The structure is ellipsoidal in shape, approximately 78 A long and 32 A wide, and comprises two domains: an alpha/beta domain (residues 1-20 and 148-230) consisting of six strands and three helices and an alpha-domain (residues 33-143) consisting of four helices. The two domains are connected by two linkers (residues 21-32 and 144-147), and in addition, at the C-terminus there is another helix which serves as a linker between the N- and C-terminal domains of intact **enzyme I**. A comparison with the recently solved X-ray structure of EIN [Liao, D.-I., Silverton, E., Seok, Y.-J., Lee, B. R., Peterkofsky, A., & Davies, D. R. (1996) Structure 4, 861-872] indicates that there are no significant differences between the solution and crystal structures within the errors of the coordinates. The active site His189 is located in a cleft at the junction of the alpha and alpha/beta domains and has a pKa of approximately 6.3. His189 has a trans conformation about chi1, a g+ conformation about chi2, and its Nepsilon2 atom accepts a hydrogen bond from the hydroxyl proton of Thr168. Since His189 is thought to be phosphorylated at the N epsilon2 position, its side chain conformation would have to change upon phosphorylation.

L11 ANSWER 26 OF 82 MEDLINE
AN 1998321639 MEDLINE
DN 98321639 PubMed ID: 9660202
TI Identification of peptides inhibiting **enzyme I** of the bacterial **phosphotransferase** system using combinatorial cellulose-bound peptide libraries.
AU Mukhija S; Germeroth L; Schneider-Mergener J; Erni B
CS Departement fur Chemie und Biochemie, Universitat Bern, Switzerland.
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1998 Jun 1) 254 (2) 433-8.
Journal code: 0107600. ISSN: 0014-2956.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199807
ED Entered STN: 19980731
Last Updated on STN: 19980731
Entered Medline: 19980721
AB The phosphoenolpyruvate(P-pyruvate)-dependent sugar phosphotransferase system (PTS) is a transport and signal-transduction system which is almost ubiquitous in bacteria but does not occur in eucaryotes. It catalyzes the uptake and phosphorylation of carbohydrates and is involved in signal transduction, e.g. catabolite repression, chemotaxis, and allosteric regulation of metabolic enzymes and transporters. EI (**Enzyme I** of the PTS) is the first and central component of the divergent PTS (P-pyruvate-dependent sugar phosphotransferase system) phosphorylation cascade. Using immobilized combinatorial peptide libraries and phosphorimaging, heptapeptides and octapeptides were identified which selectively inhibit EI in vitro. The IC50 of the best peptides is 30 microM which is close to the K(M) (6 microM) of EI for its natural substrate HPr (histidine containing phosphoryl carrier protein of the PTS). The affinity-selected peptides are better inhibitors than a peptide with the active-site sequence of HPr. The selected peptides contain several basic residues and one aromatic residue which do not occur in the active site of HPr. The large proportion of basic residues most likely reflects charge complementarity to the strongly acidic active-site pocket of EI. Guanidino groups might facilitate by complexation of the phosphoryl group the slow phosphorylation of the peptide.

L11 ANSWER 29 OF 82 MEDLINE
AN 97239066 MEDLINE
DN 97239066 PubMed ID: 9084757
TI **Enzyme I**: the first protein and potential regulator of the bacterial **phosphoenolpyruvate**: glycose **phosphotransferase** system.
AU Chauvin F; Brand L; Roseman S
CS Department of Biology, Johns Hopkins University, Baltimore, MD 21218, USA.
NC GM11632 (NIGMS)
GM38759 (NIGMS)
GM51215 (NIGMS)
SO RESEARCH IN MICROBIOLOGY, (1996 Jul-Sep) 147 (6-7) 471-9. Ref: 24
Journal code: 8907468. ISSN: 0923-2508.
CY France
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 199704
ED Entered STN: 19970422
Last Updated on STN: 19970422
Entered Medline: 19970408

L11 ANSWER 32 OF 82 MEDLINE
AN 97039713 MEDLINE
DN 97039713 PubMed ID: 8885265
TI Regulation of competence development and sugar utilization in Haemophilus influenzae Rd by a **phosphoenolpyruvate**:fructose **phosphotransferase** system.
AU Macfadyen L P; Dorocicz I R; Reizer J; Saier M H Jr; Redfield R J
CS Department of Zoology, University of British Columbia, Vancouver, Canada.. lmac@unixg.ubc.ca
NC 2RO1AI 14176 (NIAID)
5RO1AI 21702 (NIAID)
SO MOLECULAR MICROBIOLOGY, (1996 Sep) 21 (5) 941-52.
Journal code: 8712028. ISSN: 0950-382X.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199702
ED Entered STN: 19970227
Last Updated on STN: 19980206
Entered Medline: 19970211
AB Changes in intracellular cAMP concentration play important roles in Haemophilus influenzae, regulating both sugar utilization and competence for natural transformation. In enteric bacteria, cAMP levels are controlled by the phosphoenolpyruvate:glycose phosphotransferase system (PTS) in response to changes in availability of the preferred sugars it transports. We have demonstrated the existence of a simple PTS in H. influenzae by several methods. We have cloned the H. influenzae ptsI gene, encoding PTS **Enzyme I**; genome analysis locates it in a pts operon structurally homologous to those of enteric bacteria. In vitro phosphorylation assays confirmed the presence of functional PTS components. A ptsI null mutation reduced fructose uptake to 1% of the wild-type rate, and abolished fructose fermentation even when exogenous cAMP was provided. The ptsI mutation also prevented fermentation of ribose and galactose, but utilization of these cAMP-dependent sugars was restored by addition of cAMP. In wild-type cells the non-metabolizable fructose analogue xylitol prevented fermentation of these sugars, confirming that the fructose PTS regulates cAMP levels. Development of competence under standard inducing conditions was reduced 250-fold by the ptsI mutation, unless cells were provided with exogenous cAMP. Competence is thus shown to be under direct nutritional control by a fructose-specific PTS.

L7 ANSWER 4 OF 33 CAPLUS COPYRIGHT 2003 ACS
AN 1998:393650 CAPLUS
DN 129:158337
TI Identification of peptides **inhibiting enzyme I** of the bacterial **phosphotransferase** system using combinatorial cellulose-bound peptide libraries
AU Mukhija, Seema; Germeroth, Lothar; Schneider-Mergener, Jens; Erni, Bemhard
CS Departement fur Chemie und Biochemie, Universitat Bern, Bern, Switz.
SO European Journal of Biochemistry (1998), 254(2), 433-438
CODEN: EJBCAI; ISSN: 0014-2956
PB Springer-Verlag
DT Journal
LA English
AB The **phosphoenolpyruvate**(P-pyruvate)-dependent sugar **phosphotransferase** system (PTS) is a transport and signal-transduction system which is almost ubiquitous in bacteria but does not occur in eukaryotes. It catalyzes the uptake and **phosphorylation** of carbohydrates and is involved in signal transduction, e.g. catabolite repression, chemotaxis, and allosteric regulation of metabolic enzymes and transporters. EI (Enzyme I of the PTS) is the first and central component of the divergent PTS (P-pyruvate-dependent sugar **phosphotransferase** system) **phosphorylation** cascade. Using immobilized combinatorial peptide libraries and **phosphorimaging**, heptapeptides and octapeptides were identified which selectively inhibit EI in vitro. The IC50 of the best peptides is 30 .mu.M which is close to the KM (6 .mu.M) of EI for its natural substrate HPr (histidine contg. **phosphoryl** carrier protein of the PTS). The affinity-selected peptides are better inhibitors than a peptide with the active-site sequence of HPr The selected peptides contain several basic residues and one arom. residue which do not occur in the active site of HPr. The large proportion of basic residues most likely reflects charge complementarity to the strongly acidic active-site pocket of EI. Guanidino groups might facilitate by complexation of the **phosphoryl** group the slow **phosphorylation** of the peptide.
RE.CNT 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 6 OF 33 CAPLUS COPYRIGHT 2003 ACS
AN 1997:654520 CAPLUS
DN 127:328231
TI Phage display selection of peptides against enzyme I of the **phosphoenolpyruvate**-sugar **phosphotransferase** system (PTS)
AU Mukhija, Seema; Erni, Bernhard
CS Department fur Chemie und Biochemie, Universitat Bern, Bern, CH-3012, Switz.
SO Molecular Microbiology (1997), 25(6), 1159-1166
CODEN: MOMIEE; ISSN: 0950-382X
PB Blackwell
DT Journal
LA English
AB The bacterial **phosphoenolpyruvate**-sugar **phosphotransferase** system (PTS) mediates the uptake and **phosphorylation** of carbohydrates and is involved in signal transduction. In response to the availability of carbohydrates it modulates catabolite repression, intermediate metab., gene expression and chemotaxis. It is ubiquitous in bacteria but does not occur in animals and plants. Uniqueness and pleiotropic function make the PTS a target for new antibacterial drugs. Enzyme I is the first component of the divergent protein **phosphorylation** cascade of the PTS. It transfers **phosphoryl** groups from **phosphoenolpyruvate** to the general **phosphoryl** carrier protein HPr. Six 15-mer, nine 10-mer and nine 6-mer peptides that **inhibit enzyme I** were selected from phage display libraries. Of these, 16 were synthesized and characterized. The majority of the peptides contain a histidine with an adjacent arginine. Two peptides were found to contain cysteines but no histidine. All peptides are rich in basic residues and lack acidic amino acids. The peptides inhibit the **phosphotransferase** system in vitro with IC50 of between 10 .mu.M and 2 mM. Some, but not all, of the peptides inhibit cell growth in the agar diffusion test by an as yet undefined mechanism. All peptides are **phosphorylated** by enzyme I, and some are regenerated by slow autocatalytic hydrolysis of the **phospho**-peptide bond.

L7 ANSWER 14 OF 33 CAPLUS COPYRIGHT 2003 ACS
AN 1985:467412 CAPLUS
DN 103:67412
TI Evidence for covalently crosslinked dimers and trimers of enzyme I of the Escherichia coli **phosphotransferase** system
AU Grenier, Frank C.; Reizer, Jonathan; Waygood, E. Bruce; Saier, Milton H., Jr.
CS John Muir Coll., Univ. California, San Diego, La Jolla, CA, 92093, USA
SO Journal of Bacteriology (1985), 163(1), 243-7
CODEN: JOBAAY; ISSN: 0021-9193
DT Journal
LA English
AB Enzyme I of the bacterial **phosphotransferase** system catalyzes transfer of the **phosphoryl** moiety from PEP to both of the heat-stable **phosphoryl** carrier proteins of the **phosphotransferase** system, HPr and FPr. By using SDS-polyacrylamide gel electrophoresis and HPLC, the existence of covalently crosslinked enzyme I dimers and trimers was demonstrated. Enzyme I exchange assays and **phosphorylation** expts. with [32P]PEP showed that covalent dimers and trimers are catalytically active. **Inhibitors** of the **enzyme I**-catalyzed PEP-pyruvate exchange block the **phosphorylation** of enzyme I dimers and trimers. Inhibition of the activity of enzyme I by N-ethylmaleimide, but not that by p-chloromercuriphenylsulfonate, could be overcome by high concns. of enzyme, suggesting that N-ethylmaleimide modification changes the associative properties of enzyme I. Two distinct classes of SH groups exist in enzyme I.

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AN 1980:600056 CAPLUS
DN 93:200056
TI **Phosphoryl** exchange reaction catalyzed by enzyme I of the bacterial **phosphoenolpyruvate**:sugar **phosphotransferase** system. Kinetic characterization
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SO Journal of Biological Chemistry (1980), 255(18), 8579-84
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB Enzyme I (EC 2.7.3.9) of the bacterial **phosphoenolpyruvate**-sugar **phosphotransferase** system catalyzes **phosphoryl** transfer from **phosphoenolpyruvate** (PEP) to the heat-stable **phosphoryl** carrier protein, HPr. Enzyme I also catalyzes a rapid **phosphoryl** exchange reaction in which the **phosphoryl** moiety of PEP is transferred to pyruvate. Addnl., Enzyme I plus HPr catalyze the slow hydrolysis of PEP to pyruvate and inorg. **phosphate**. A simple and quant. assay was developed for measuring these reactions. 14C-labeled PEP or pyruvate was used, and the formation of pyruvate-14C or PEP-14C was measured, resp. The 2 radioactive products were sepd. by conversion of pyruvate to the dinitrophenylhydrazone deriv. and extn. into EtOAc. Employing this assay, the enzyme I-catalyzed **phosphoryl** exchange and sugar **phosphorylation** reactions showed sigmoidal kinetics when the reaction rate was plotted vs. enzyme I concn. The enzyme was activated by Mg2+, Mn2+, and Co2+, but not by other divalent cations tested. The pH optimum in the presence of Mg2+ was 7.5. Kinetic binding consts. were estd. as follows: PEP 0.4 mM; pyruvate, 2 mM; Mg2+, 2 mM; PEP (bound to **phospho**-enzyme I), 2.5 mM; and pyruvate (bound to free enzyme I), 20 mM. Low concns. of bromopyruvate irreversibly inactivated the enzyme, whereas oxalate appeared to be a potent transition state inhibitor. Enzyme I catalyzed exchange reactions when .alpha.-ketobutyrate or .beta.-hydroxypyruvate replaced pyruvate, but higher homologs of pyruvate were essentially inactive. Bacterial exts. catalyzed the hydrolysis of PEP in the presence of low concns. of Co2+. This activity was not attributable to a known enzyme constituent of the **phosphotransferase** system.